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MAY 21 2007

Appl. No. 10/626,493
Amtd. dated May 21, 2007
Reply to Office Action of March 1, 2007,

PATENT

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings of claims in the application:

Listing of Claims:

1. (Currently amended) A method for creating a profile of interactions between components of at least one multicomponent biological complex in a sample, the method comprising, for each complex:

(a) providing an aliquot from the sample, wherein the aliquot comprises the multicomponent biological complex;

~~(b) immobilizing the multicomponent biological complex from the sample~~
immobilized on a solid support through a biospecific affinity molecule, wherein the affinity molecule is not a nucleic acid, and wherein the affinity molecule binds a first component of the complex and wherein unbound material has been removed from the solid support;

~~(b)(c)~~ washing the immobilized multicomponent biological complex with a sequence of elution washes, wherein a first solute in each elution wash, has a concentration such that the sequence of elution washes forms a gradient of increasing or decreasing concentration of the first solute; and

~~(c)(d)~~ measuring for a second component in each of the elution washes; whereby the profile for the complex from the sample comprises the measurements from the elution washes.

2. (Previously presented) The method of claim 1 wherein the sample is selected from the group consisting of tissue extracts, cell extracts, blood, urine, lymphatic fluid, *in vitro* protein expression media and derivatives thereof.

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3. (Original) The method of claim 1 wherein the at least one complex is one complex.
4. (Original) The method of claim 1 wherein the at least one complex is a plurality of complexes, each bound through a biospecific affinity reagent.
5. (Currently amended) The method of claim 1 wherein the affinity molecule is selected from the group consisting of: an antibody, a single chain antibody, a specific binding fragment of an antibody, an affibody, an enzyme, an enzyme substrate, a receptor, a receptor ligand, a drug, ~~a nucleic acid, or~~ and an aptamer.
6. (Original) The method of claim 1 wherein the affinity molecule is immobilized to the solid support before binding the complex.
7. (Original) The method of claim 1 wherein the affinity molecule is bound to the solid support after binding the complex.
8. (Original) The method of claim 1 wherein the solid support is a chromatographic resin.
9. (Original) The method of claim 8 wherein the washes are performed in a non-flow-through device.
10. (Original) The method of claim 9, wherein the non-flow through device is a closed bottomed microtiter plate.
11. (Original) The method of claim 8 wherein the washes are performed in a flow-through device.
12. (Original) The method of claim 11 wherein the flow-through device is a microtiter drip plate, a flow-through column or a flow-through microcolumn.

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13. (Previously presented) The method of claim 1 wherein the solid support is a SELDI probe comprising the biospecific affinity molecule attached to the probe surface for capturing the complex.

14. (Original) The method of claim 1 wherein the unbound material is removed with an initial wash.

15. (Original) The method of claim 1 wherein the solute is selected from an ion, a salt, a detergent, a biomolecule or a binding competitor.

16. (Previously presented) The method of claim 1, further comprising washing the immobilized multicomponent biological complex in a second aliquot of the sample with a second sequence of elution washes, wherein the second solute is different than the first solute.

17. (Original) The method of claim 1 wherein the second component is detected by an optical method, an electrochemical method, atomic force microscopy or a radio frequency method.

18. (Original) The method of claim 1 wherein the second component is detected by mass spectrometry.

19. (Original) The method of claim 18 wherein mass spectrometry is affinity mass spectrometry.

20. (Original) The method of claim 19 wherein affinity mass spectrometry comprises SEND.

21. (Currently amended) The method of claim 1 further comprising after step (b) (c), measuring components of the complex still immobilized on the support through the biospecific affinity molecule, whereby the profile further comprises the measurements of the complex.

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22. (Currently amended) A method comprising:

a. (a) providing a set of biological samples, wherein the set comprises at least two subsets, each subset characterized by a different biological characteristic;

b. (b) creating a profile of interactions between components of at least one multicomponent biological complex for each sample in the set, wherein the method comprises:

(i) providing an aliquot from each sample, wherein the aliquot comprises the multicomponent biological complex;

(ii) immobilizing the multicomponent biological complex from the sample
~~immobilized~~ on a solid support through a biospecific affinity molecule, wherein the affinity molecule is not a nucleic acid, and wherein the affinity molecule binds a first component of the complex and wherein unbound material has been removed from the solid supports;

~~(ii)~~ (iii) washing the immobilized complex with a plurality of successive elution washes, wherein the concentration of a solute in the successive elution washes form a gradient of increasing or decreasing concentration; and

~~(iii)~~ (iv) measuring a second component in the successive elution washes, whereby the profile for each sample in the set comprises the measurements from the elution washes from each aliquot; and

c. (c) comparing the profiles for each sample in the set to detect differences in interaction between components in each subset.

23. (Currently amended) The method of claim 22 wherein the different biological characteristics are selected from the pairs consisting of: pathological v. and non-

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pathological, drug responder ~~v.~~ and drug non-responder, toxic response ~~v.~~ and non-toxic response, and progressor to disease state ~~v.~~ and non-progressor to disease state.

24. (Previously presented) The method of claim 22 further comprising exposing the samples to be compared to different conditions, wherein one sample is exposed to an inhibitor RNA and the other sample is not exposed to the inhibitor RNA.

25. (Currently amended) The method of claim 22 wherein step (b) further comprises, after step ~~(ii)~~ (iii) detecting components of the complex still immobilized on the support through the biospecific affinity molecule, whereby the profile further comprises the measurements from the support.

26. (Currently amended) The method of claim 22, further comprising performing steps (b)(i)-~~(iii)~~(iv) on a second aliquot from each samples in the set, wherein the elution washes comprise a second, different solute and the concentrations of the second solute in the successive elution washes form a gradient of increasing or decreasing concentration.

27. (Original) The method of claim 22 wherein comparing comprises using the profiles to train a computerized learning algorithm, wherein the computerized learning algorithm generates a classification algorithm that classifies a profile into one of the at least two subsets.

28. (Currently amended) A method for creating a profile of interactions between components of at least one multicomponent biological complex in a sample, the method comprising, for each complex:

(a) providing a plurality of aliquots from the sample, each aliquot comprising the same multicomponent biological complex;

(b) immobilizing the multicomponent biological complex from the sample
~~immobilized~~ on a solid support through a biospecific affinity molecule, wherein the affinity

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molecule is not a nucleic acid and wherein the affinity molecule binds a first component of the complex and wherein unbound material has been removed from the solid supports;

~~(b)~~(c) washing the immobilized multicomponent biological complex in each of the aliquots with a sequence of elution washes, wherein a first solute in each elution wash has a concentration such that the sequence of elution washes forms a gradient of increasing or decreasing concentration of the first solute; and

~~(e)~~(d) measuring at least one second component in each of the elution washes; whereby the profile for the sample comprises the measurements from the elution washes from each aliquot.

29. (Currently amended) The method of claim 28 further comprising after step ~~(b)~~(c), detecting components of the complex still immobilized on the support through the biospecific affinity molecule, whereby the profile further comprises the measurements from the support.

30. (Currently amended) The method of claim 28 further comprising performing step ~~(b)~~(c) on a second plurality of aliquots from the sample, wherein the elution washes comprise a second, different solute and the concentrations of the second solute in the successive elution washes form a gradient of increasing or decreasing concentration.

31. (Currently amended) A method comprising:

(a) providing a set of biological samples, wherein the set comprises at least two subsets, each subset characterized by a different biological characteristic;

(b) creating a profile of interactions between components of at least one multicomponent biological complex for each sample in the set, wherein creating a profile for a complex in a sample comprises:

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(i) providing a plurality of aliquots from each sample in the set, each aliquot comprising the same multicomponent biological complex;

~~(ii) immobilizing the multicomponent biological complex from the sample~~ immobilized on a solid support through a biospecific affinity molecule, wherein the affinity molecule is not a nucleic acid and wherein the affinity molecule binds a first component of the complex and wherein unbound material has been removed from the solid supports;

~~(ii)~~(iii) washing the immobilized complex in each of the aliquots with an elution wash of a first sequence of elution washes, wherein the concentrations of a first solute in the elution washes of the sequence form a gradient of increasing or decreasing concentration; and

~~(iii)~~(iv) measuring at least one second component in each of the elution washes; whereby the profile for a complex in the set comprises the measurements from the elution washes from each aliquot; and

(c) comparing the profiles for the samples to detect differences in interaction between components in the samples.

32. (Previously presented) The method of claim 31 further comprising washing the immobilized complex in a second plurality of aliquots from each sample with one elution wash of a second set of elution washes, wherein the concentrations of a second solute in each member of the set of elution washes form a gradient of increasing or decreasing concentration, and wherein the second solute is different than the solute.

33. (Currently amended) The method of claim 31 wherein step (b) further comprises, after step ~~(ii)~~(iii) detecting components of the complex still immobilized on the support through the biospecific affinity molecule, whereby the profile further comprises the measurements from the support.

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34. (Currently amended) The method of claim 31 further comprising performing steps (b), (i)-~~(iv)~~~~(iii)~~ on a second plurality of aliquots from the set, wherein the elution washes comprise a second, different solute and the concentrations of the second solute in the successive elution washes form a gradient of increasing or decreasing concentration.

35. (Previously presented) The method of claim 32 wherein comparing the profiles for the set detects differences in interaction between components in the samples.

36-53. (Cancelled)